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Biotransformation enzyme expression in the nasal epithelium of woodrats

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ABSTRACT

When herbivores come in contact with volatile plant secondary compounds (PSC) that enter the nasal passages the only barrier between the nasal cavity and the brain is the nasal epithelium and the biotransformation enzymes present there. The expression of two biotransformation enzymes Cytochrome P450 2B (CYP2B) and glutathione-S-transferase (GST) was investigated in the nasal epithelia and livers of three populations of woodrats. One population of *Neotoma albigula* was fed juniper that contains volatile terpenes. Juniper caused upregulation of CYP2B and GST in the nasal epithelium and the expression of CYP2B and GST in the nasal epithelium was correlated to liver expression, showing that the nasal epithelia responds to PSC and the response is similar to the liver. Two populations of *Neotoma bryanti* were fed creosote that contains less volatile phenolics. The creosote naive animals upregulated CYP2B in their nasal epithelia while the creosote experienced animals upregulated GST. There was no correlation between CYP2B and GST expression in the nasal epithelia and livers of either population. The response of the nasal epithelium to PSC seems to be an evolved response that is PSC and experience dependent.

1. Introduction

A foraging animal must make decisions about when to eat, what to eat, and how much to consume. For herbivores, these decisions are further complicated by having to cope with the possibility of being poisoned by their food. Plants defend themselves against herbivory in a number of ways mechanical, chemical and phenological (Stamp, 2003). Chemical defenses using plant secondary compounds (PSC) can be a major deterrent of predation as these toxic compounds can lead to weight loss, liver damage, and in severe cases death of herbivores (Freeland and Janzen, 1974; Dearing et al., 2005a). However, herbivores have developed effective strategies to feed on these plants without jeopardizing health.

These strategies can be as simple as reducing intake of the plant or as complex as enzymatic reactions, which biotransform and assist in excretion of the PSC from the body (Freeland and Janzen, 1974; Sorensen and Dearing, 2003; Dearing et al., 2005a; Sorensen et al., 2006; Glendinning, 2007; Torregrossa and Dearing, 2009; Torregrossa et al., 2011; Torregrossa et al., 2012). In vertebrates the greatest proportion of biotransformation of ingested xenobiotic substances is carried out by the liver, kidneys, and intestines (Klaassen and Watkins, 2003). During feeding however, foreign substances can also enter the body through the nasal cavity. The nasal passages lie in close proximity to the brain and the two are separated only by nasal epithelium, as the blood–brain-barrier is absent in this region. The nasal epithelium along with mucous provide a barrier against inhaled particles and previous studies have shown that the nasal epithelium possesses several biotransformation enzymes that respond to inhaled toxins (Thornton-Manning and Dahl, 1997; Minn et al., 2002; Ling et al., 2004; Minn et al., 2005; Thiebaud et al., 2010). However, the role of the nasal epithelium in processing volatile PSC has never been investigated in wild-caught herbivores. We compared the biotransformation enzyme expression in three populations of woodrats: *Neotoma albigula* was fed juniper (*Juniperus osteosperma*), and two populations of *Neotoma bryanti* were fed creosote bush resin (*Larrea tridentata*). The two populations of *N. bryanti* represented creosote naive and creosote experienced animals.

Terpenes, the major class of PSC in juniper (Adams, 2000), are highly volatile, neurotoxic compounds (Sperling et al., 1967; Sperling, 1969; Savolainen and Pfaffli, 1978; Falk et al., 1990) that are found in the brain after inhalation (Satou et al., 2012). Juniper does contain other less volatile PSC like phenolics however they make up a small fraction of the essential oil present (Adams et al., 2007). Since *N. albigula* consumes up to 30% of their diet as juniper in the wild (Dial, 1988), we expect that *N. albigula*'s nasal epithelium has evolved defenses to the volatile terpenes present in juniper and therefore will respond to juniper feeding by upregulating biotransformation enzymes responsible for metabolizing terpenes (Haley et al., 2007b; Skopec et al., 2007).

The major class of PSC in creosote is phenolics (Cameron and Rainey, 1972; Karasov, 1989; Meyer and Karasov, 1989). Creosote naive animals were trapped in coastal southern California, an area

Abbreviations: PSC, Plant secondary compounds; CYP2B, Cytochrome P450 2B; GST, Glutathione-S-transferase.

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that does not contain creosote. Creosote experienced animals were trapped in the Mojave desert near Barstow California, and consume up to 75% of their diet as creosote in the wild (Cameron and Rainey, 1972; Karasov, 1989). We fed the two populations of *N. bryanti* creosote to determine if the response of the nasal epithelium to PSC is a generalized or evolved response by comparing the response of biotransformation enzymes in the nasal epithelium of the creosote naive population of *N. bryanti* to the creosote experienced population of *N. bryanti*.

We examined the levels of two biotransformation enzymes, Cytochrome P450 2B (CYP2B) and glutathione-S-transferase (GST). Both CYP2B and GST are known to occur in the nasal epithelium in a number of species (Sarkar, 1992; Ben-Arie et al., 1993; Thornton-Manning and Dahl, 1997; Ding and Kaminsky, 2003) and are important for the metabolism of the PSC present in juniper and creosote bush (Haley et al., 2007b; Skopec et al., 2007; Haley et al., 2008; Magnanou et al., 2009). CYP2B is a phase I or functionalization enzyme that oxidizes its substrate aiding further metabolism, while GST is a phase II or conjugation enzyme that functions by catalyzing the addition of glutathione group to its substrate, aiding excretion.

We tested three hypotheses. The first hypothesis is that biotransformation enzymes in the nasal epithelium will respond to PSC in the diet. The second hypothesis is that the biotransformation enzymes upregulated in the nasal epithelium will be similar to those upregulated in the liver. We addressed these hypotheses by examining the protein expression of CYP2B and GST in the nasal epithelia, olfactory bulbs and livers of two species of woodrats consuming different PSC common to their natural diets. The third hypothesis is that the response of biotransformation enzymes in the nasal epithelium is an evolved response and woodrats exposed to novel PSC will have a different response than those exposed to PSC they have evolutionary experience with. To address this we examined the protein expression, as described above, of two populations of woodrats on PSC diet common to one population but novel to the other.

2. Materials and methods

2.1. Woodrats

Neotoma albigula was trapped in Castle Valley, Utah (38°30′N, 109°18′W). One population of *N. bryanti* was captured near Caspers Wilderness Park in Orange County, CA, USA (33°31′N, 117°33′W). This area of California does not contain creosote and *N. bryanti* trapped from this area are naive to feeding on creosote. Individuals from a second population of *N. bryanti* were collected near Boyd Deep Canyon Reserve near Palm Springs, CA, USA (33°40′N, 116°22′W), which is in the Mojave desert and contains creosote. *N. bryanti* from Palm Springs have previous experience feeding on creosote. All woodrats were transported to the University of Utah, Department of Biology's Animal Facility. Woodrats were housed in individual cages (48×27×20 cm) with pine shavings. Environmental conditions were 12:12 h light: dark cycle, ambient temperature of 28 °C and humidity of 15%. All experimental procedures involving woodrats were approved by the University of Utah's Institutional Animal Care and Use Committee protocol number 0702015.

2.2. Dietary treatments

Neotoma albigula were either fed a non-toxic control diet (Harlan Teklad high fiber rabbit chow 2031, n = 7), or a 30% juniper diet (30% ground juniper and 70% rabbit chow, on a dry matter basis, n = 6) for >5 days. The animals fed the juniper diet were in a separate room from the animals fed the control diet to prevent the control animals from smelling the juniper. Juniper was collected from trees at woodrat trapping sites, crushed on dry ice until it passed through a 1.0 mm screen and kept frozen at −20 °C until use. All diets were mixed daily to minimize volatilization of the terpenes. Food intakes and body weights were measured daily.

N. bryanti were fed either the control diet (n = 5 creosote naive, n = 5 creosote experienced) or a 2% creosote resin diet (n = 4 creosote naive, n = 5 creosote experienced) for 5 days. The creosote diet consisted of 2% creosote resin in rabbit chow, on a dry matter basis. Creosote bush leaves were collected from woodrat trapping sites and kept frozen at −20 °C. Resin was extracted and creosote diets were prepared as in Magnanou et al. (2009). Food intakes and body weights were measured daily.

We chose to not feed juniper to *N. bryanti or* creosote to *N. albigula* because we believed these would be ecologically irrelevant comparisons and interpreting the data would be difficult in light of the documented species differences in biotransformation (Haley et al., 2007a,b, 2008; Skopec et al., 2007; Skopec and Dearing, 2011). Neither population of *N. bryanti* currently consumes juniper. Creosote bush (*L. tridentata*) replaced juniper ca. 17,000 years ago in the Mojave during a natural climatic event (Van Devender, 1977; Van Devender and Spaulding, 1979) so although the creosote experienced population may have evolved from juniper feeding ancestors they have no ecological experience with it. The creosote naive population from southern California lives in a juniper free environment and consumes a diet of cactus and oak (Skopec et al., 2008) and therefore has no current or evolutionary exposure to juniper. Likewise, *N. albigula* have no current or evolutionary experience with creosote. We chose to test our hypothesis that woodrats exposed to novel PSC will have a different response than those exposed to PSC they have evolutionary experience with by feeding creosote to a population of *N. bryanti* that we believe to be creosote naive because a within species comparison allows us to test the hypothesis with more closely related groups of animals.

2.3. Tissue harvesting and sample preparation

On the last day of the feeding trial animals were euthanized with an overdose of CO_2 and the nasal epithelia, olfactory bulbs and liver tissue were harvested. Animals were decapitated and the heads rinsed with 0.9% NaCl saline solution. The skull was exposed and a scalpel was used to make an incision along the rostrum, then force was applied to separate the skull into two halves. The nasal epithelia and olfactory bulbs of each animal were then collected with the use of a scalpel and forceps and flash frozen and stored at −80 °C. Samples were homogenized using a glass homogenizer and 50 mmol Na_3PO_4 (trisodium phosphate) buffer in a 10 μL:1 μg ratio. Cytosolic and membrane fractions were prepared for both the nasal epithelium and olfactory bulb samples via centrifugation at 20,000 g for 120 min at 4 °C. The supernatant was saved as the cytosolic fraction. The pellet was washed twice with a 50 mmol Na_3PO_4 buffer and re-suspended, as the membrane fraction. The fractions were frozen at −80 °C until the assays were performed.

Livers were perfused in situ with cold isotonic saline via the hepatic portal vein, extracted and weighed. In order to isolate tissue specific enzymes, microsomal and cytosolic fractions were created from the liver by differential ultracentrifugation as described for laboratory rats by Franklin and Estabrook (1971). Samples were stored at −80 °C until use. Protein concentrations for all samples were determined colorimetrically via the Bio-Rad Protein assay (Bio-Rad) based on the Bradford dye-binding method (Kruger and Walker, 2002).

2.4. Western blot and chemiluminescence imaging

Samples were diluted to 1.25 μg/μL with 1 M Tris HCl pH 7.4, placed in loading buffer (4% SDS, 20% glycerol, 0.1% bromophenol blue, 250 mM Tris HCl pH 6.9, 0.2% 2-beta mercaptoethanol) and denatured by heating at 100 °C for 3 min. 25 μg of protein was loaded into each well of a 4–20% Tris glycine iGel (ISC bioexpress) and the samples were subjected to SDS-polyacrylamide gel electrophoresis

and then transferred onto polyvinylidene difluoride (PVDF) membranes (Thermo Scientific). The membranes were blocked for 1 h using 5% skim milk in Tris-buffered saline with 0.01% Tween and then incubated with the primary antibodies. The membrane and microsomal samples were incubated with polyclonal rabbit anti-rat CYP2B (1:1000 (provided by Dr. James Halpert, UC San Diego, CA, USA) known to cross react with rat, mouse and woodrat) and the cytosolic samples were incubated with polyclonal goat anti-rat GST-Ya (1:1000 (US Biological) known to cross react with human, mouse and woodrat). The blots were visualized with peroxidase labeled goat anti-rabbit or rabbit anti-goat secondary antibodies (1:10,000 (KPL)) and Pierce ECL Western Blotting Substrate (Thermo Scientific). A Typhoon 8600 (Molecular Dynamics 300-2483) imaging system using the following setting (200 μm pixel size, normal limit of detection and PMT voltage se at 800 V) was used to visualize chemiluminescence on the blots. ImageQuant software was used to quantify protein bands on the membranes.

All *N. albigula* samples were normalized to a common reference sample that was run on all gels containing *N. albigula* samples. All *N. bryanti* samples were normalized to a common reference sample that was run on all gels with *N. bryanti* samples. Samples were run in duplicate and the average band volume for each sample was used in the analysis.

2.5. Statistical analysis

Body mass, dry matter intake and liver masses of *N. albigula* were compared using *t*-tests. Body mass of *N. bryanti* was analyzed using two-factor analysis of variance (ANOVA) with population and treatment as factors. Dry matter intake of *N. bryanti* was analyzed using two-factor analysis of covariance (ANCOVA) with population and treatment as factors and body mass as the covariate because there was a significant difference in the body masses of the two populations. Liver masses of *N. bryanti* were also analyzed using two-factor ANCOVA with population and treatment as factors but with body mass minus organ mass as the covariate (Christians, 1999). For all ANOVAs and ANCOVAs post-hoc Bonferroni adjusted pairwise comparisons were used to determine differences between individual means. Nasal epithelia and olfactory bulbs were not weighed due to small sample size. Enzyme expression was expressed relative to the reference sample for each species *N. albigula* and *N. bryanti* and was compared within species and tissue. *t*-tests were used to determine differences in CYP2B and GST expression in the nasal epithelium and liver of *N. albigula*. For *N. bryanti*, two-factor ANOVAs with population and treatment as factors were used to determine differences in CYP2B and GST expression in the nasal epithelium and liver: differences between individual means were determined by post-hoc Bonferroni adjusted pairwise comparisons. None of the olfactory bulb samples expressed either CYP2B or GST so statistical analysis was not conducted. To analyze the correlation between nasal epithelium and liver expression of CYP2B and GST enzyme expression was log transformed and general linear models (GLM) were used. For *N. albigula* nasal epithelium expression of CYP2B or GST was used as the dependent variable and liver expression of the same enzyme and treatment were used as factors. For *N. bryanti*, nasal epithelium was also the dependent variable and liver expression, population, and treatment were used as factors. SYSTAT 10 was used for all analyses (Wilkinson and Coward, 2000). All data are expressed as mean ± 1 SE and $p \leq 0.05$ was used to establish significance.

3. Results

3.1. Body mass, dry matter intake and liver mass

Body mass, dry matter intake and liver mass did not differ between *N. albigula* fed the control or juniper diet ($T = 0.167$, $df = 11$, $p = 0.87$ for body mass, $T = 0.139$, $df = 11$, $p = 0.89$ for dry matter intake and $T = 1.762$, $df = 11$, p-0.11 for liver mass, Table 1).

The two populations of *N. bryanti* differed in body mass ($F_{1,15} = 9.607$, $p = 0.007$, Table 2). The creosote experienced animals fed the creosote diet were significantly smaller than the creosote naive animals fed the control diet ($p = 0.002$). There was no effect of treatment ($F_{1,14} = 0.148$, $p = 0.705$) or interaction between population and treatment ($F_{1,15} = 0.032$, $p = 0.860$) meaning all animals fed the creosote diets maintained body mass similar to their respective controls.

Dry matter intake did not differ between the *N. bryanti* populations ($F_{1,14} = 2.487$, $p = 0.137$) and body mass was not a significant covariate ($F_{1,14} = 1.330$, $p = 0.268$). There was a significant treatment effect on dry matter intake ($F_{1,14} = 14.326$, $p = 0.002$) and a significant interaction between population and treatment ($F_{1,14} = 11.447$, $p = 0.004$). The creosote naive animals fed the creosote diet had significantly lower dry matter intakes than both populations of *N. bryanti* fed the control diet (all ps<0.008). However, dry matter intake of the creosote diet did not differ between the two populations ($p = 0.13$).

Liver mass varied between *N. bryanti* populations ($F_{1,14} = 7.869$, $p = 0.014$) and body mass minus liver mass was a significant covariate ($F_{1,14} = 67.536$, $p < 0.001$). There was no effect of treatment on liver mass ($F_{1,14} = 0.280$, $p = 0.605$) and no interaction between population and treatment ($F_{1,14} = 1.071$, $p = 0.318$). Post-hoc Bonferroni adjusted pairwise comparisons revealed no significant differences between means, meaning the body mass differences between the populations were driving liver mass differences i.e., larger animals had larger livers.

3.2. CYP 2B and GST in N. albigula

When consuming the juniper diet, *N. albigula* increased the expression of CYP2B in the nasal epithelium ($t = -2.517$, $df = 10$, $p = 0.031$, Fig. 1) but not in the liver ($t = -0.806$, $df = 9$, $p = 0.440$). The *N. albigula* consuming the juniper diet increased the expression of GST in both the nasal epithelium ($t = -2.625$, $df = 7$, $p = 0.034$, Fig. 2) and liver ($t = -4.809$, $df = 7$, $p = 0.002$).

3.3. CYP2B and GST in N. bryanti

The creosote naive population of *N. bryanti* consuming the control diet expressed less CYP2B in the nasal epithelium than the other groups of *N. bryanti* (all ps<0.05, Fig. 3). This led to a significant population ($F_{1,13} = 4.829$, $p = 0.047$) and treatment effect ($F_{1,13} = 6.673$, $p = 0.023$) as well as a significant interaction between population and treatment ($F_{1,13} = 5.038$, $p = 0.043$) for CYP2B expression in the nasal epithelium. There were no population ($F_{1,14} = 1.086$, $p = 0.315$) or diet differences ($F_{1,14} = 0.289$, $p = 0.512$) in the expression of CYP2B in the livers of *N. bryanti*.

The creosote-experienced population of *N. bryanti* had greater expression of GST in the nasal epithelium compared to the creosote experienced *N. bryanti* consuming the control diet (Fig. 4). There was no effect of diet in the creosote naive population but creosote naive woodrats had higher expression of GST in the nasal epithelium when feeding on the creosote diet than the creosote experienced *N. bryanti* consuming the control diet. This led to a significant treatment effect ($F_{1,13} = 59.248$, $p < 0.01$) and a significant interaction between treatment and population ($F_{1,13} = 12.089$, $p = 0.004$) for GST in the nasal epithelium. In the liver, the creosote naive *N. bryanti*

Table 1
Means ± SE body mass, dry matter intake, and liver mass for *Neotoma albigula*.

	Neotoma albigula	
Variable	Control diet (n = 7)	Juniper diet (n = 6)
Body mass (g)	176.66 ± 7.54	178.78 ± 10.26
Dry matter intake(g/day)	14.19 ± 1.06	14.41 ± 1.19
Liver mass (g)	7.03 ± 0.83	9.18 ± 0.90

Table 2
Means ± SE body mass, dry matter intake and liver mass for *Neotoma bryanti*.

	Neotoma bryanti			
	Creosote experienced		Creosote naive	
Variable	Control diet (n=5)	Creosote diet (n=5)	Control diet (n=5)	Creosote diet (n=4)
Body mass (g)	127.96 ± 12.02[a,b]	120.28 ± 9.09[b]	161.48 ± 11.95[a]	159.05 ± 14.35[a,b]
Dry matter intake (g/day)	9.24 ± 0.50[a]	8.90 ± 0.59[a,b]	10.54 ± 0.68[a]	6.32 ± 0.52[b]
Liver mass (g)	4.20 ± 0.60	3.66 ± 0.23	4.54 ± 0.39	4.55 ± 0.58

[a,b]Different letters (a, b) denote means significantly different ($p \leq 0.05$) as determined by Bonferroni adjusted pairwise comparisons within the *Neotoma bryanti* values in the same row.

had significantly greater expression of GST than the creosote experienced *N. bryanti* consuming the creosote diet (Fig. 4). This led to a significant population effect ($F_{1,13} = 12.668$, $p = 0.003$) for GST in the liver. There was no treatment effect ($F_{1,15} = 0.002$, $p = 0.963$) and no interaction between population and treatment ($F_{1,15} = 1.798$, $p = 0.200$) on the expression of GST in the liver.

3.4. Correlation of CYP2B and GST in the nasal epithelium compared to the liver

In *N. albigula* there is a strong correlation between the expression of CYP2B and GST in the liver compared to the nasal epithelium (Figs. 5 and 6). Liver expression of CYP2B significantly predicts nasal epithelium expression ($F_{1,8} = 73.812$, $p < 0.001$) and there is no difference between the two diet treatments ($F_{1,8} = 2.200$, $p = 0.176$). Liver expression of GST also significantly predicts nasal epithelium expression ($F_{1,5} = 15.970$, $p = 0.01$) but there is a difference between the two diet treatments ($F_{1,5} = 7.940$, $p = 0.037$). *N. albigula* consuming the juniper diet have a significant correlation between the liver and the nasal epithelium expression of GST ($p = 0.047$) while the *N. albigula* consuming the control diet did not ($p = 0.138$).

There is no correlation between the liver and the nasal epithelium expression of CYP2B ($F_{1,12} = 0.662$, $p = 0.432$) or GST ($F_{1,12} = 3.021$, $p = 0.108$) in *N. bryanti* (Figs. 7 and 8).

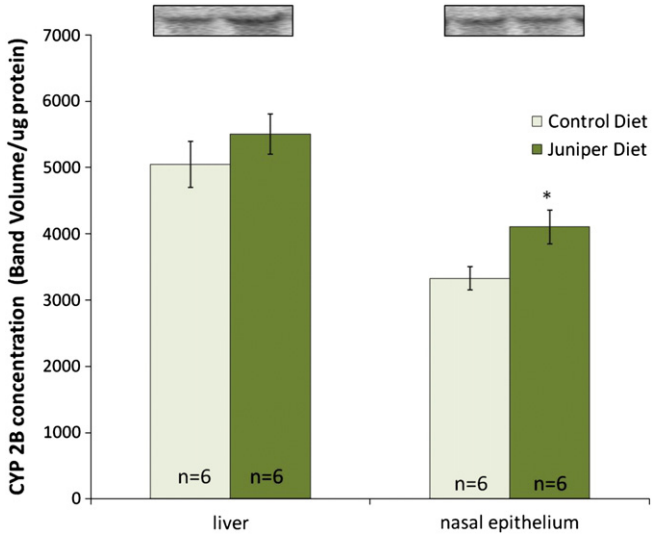

Fig. 1. The liver and the nasal epithelium CYP2B expression in *N. albigula* fed a control diet or 30% juniper diet. Western blot representative bands from each group are shown at the top of the figure. Bars show mean ± SE. * denotes means significantly different within an organ $p < 0.05$.

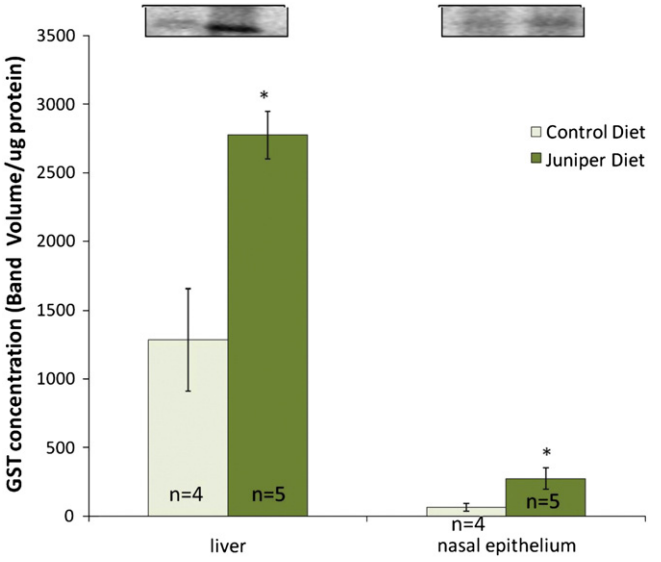

Fig. 2. The liver and the nasal epithelium GST expression in *N. albigula* fed a control diet or 30% juniper diet. Western blot representative bands from each group are shown at the top of the figure. Bars show mean ± SE. * denotes means significantly different within an organ $p < 0.05$.

4. Discussion

Potentially toxic PSC can gain entry into herbivores either through the gastrointestinal tract via ingestion or through the nasal passages where the nasal epithelium is the only barrier between the nasal cavity and the brain. The role of the nasal epithelium in protecting wild mammalian herbivores has not been investigated to date. We compared the expression of two biotransformation enzymes known to play a role in the metabolism of PSC, in three populations of woodrats,

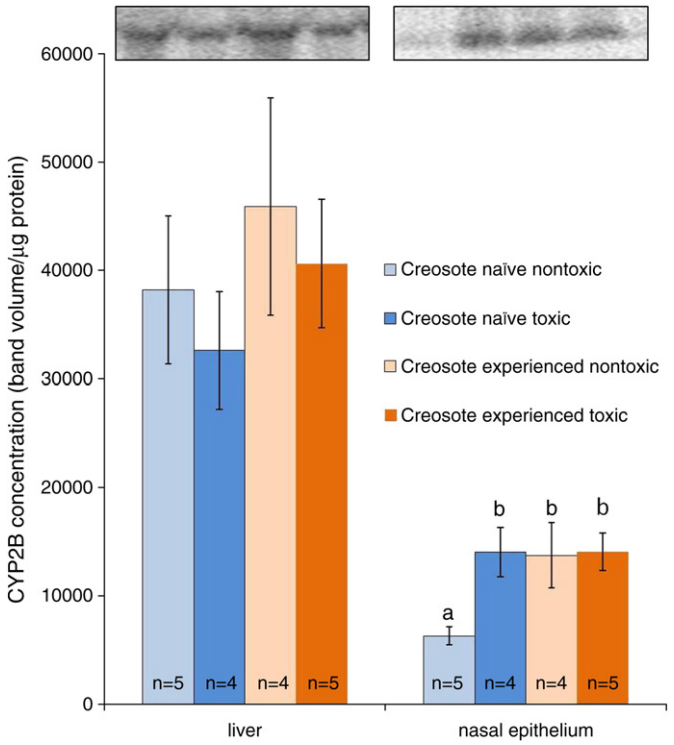

Fig. 3. The liver and the nasal epithelium CYP2B expression in *N. bryanti* that were creosote naïve or creosote experienced fed a control or a 2% creosote resin diet. Western blot representative bands from each group are shown at the top of the figure. Bars show mean ± SE. [a,b] denotes means significantly different within an organ $p < 0.05$.

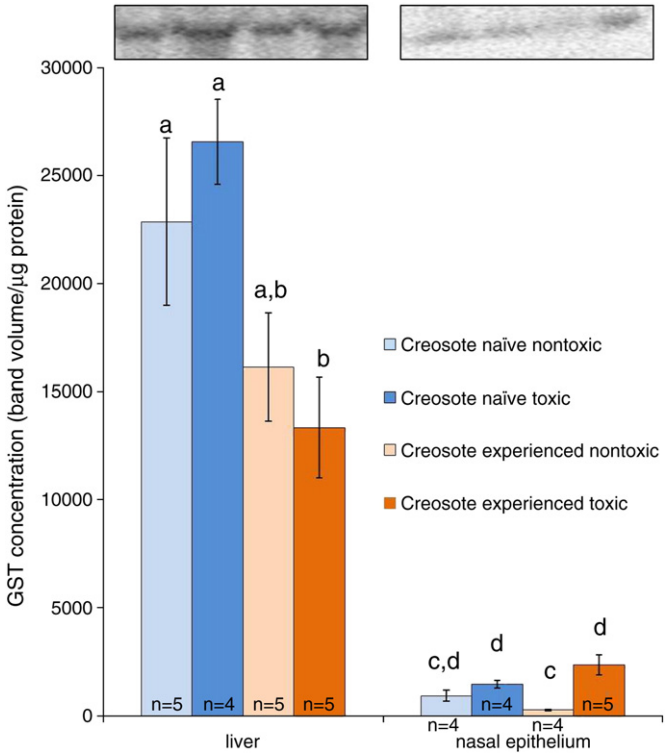

Fig. 4. The liver and the nasal epithelium GST expression in *N. bryanti* that were creosote naïve or creosote experienced fed a control or a 2% creosote resin diet. Western blot representative bands from each group are shown at the top of the figure. Bars show mean ± SE. ^{a,b,c,d} denotes means significantly different within an organ $p<0.05$.

consuming either control or toxic diets. We found marked differences in the response of the nasal epithelium to PSC in the three populations and demonstrated that the response is both PSC and experience dependent.

4.1. Does the nasal epithelium respond to PSC?

All three populations of woodrats tested showed expression of CYP2B and GST in their nasal epithelia but not in their olfactory bulbs (data not shown). The expression of CYP2B and GST in the nasal epithelium varied across populations depending on treatment and evolutionary experience with the PSC, but the overall protective nature of the nasal epithelium is common to all three populations. *Neotoma albigula* responded to juniper consumption with an upregulation of GST and CYP2B, whereas *N. bryanti* responded in a population dependant manner with either GST or CYP2B. We, therefore, conclude that the nasal epithelium is protecting wild herbivores from inhaled PSC but in a population and PSC dependent manner. Juniper contains terpenes that are highly volatile and known neurotoxins therefore increasing both phase I (CYP2B) and phase II (GST) mechanisms to metabolize the PSC in juniper may be vital for *N. albigula*'s ability to consume juniper without signs of neurotoxicity. Relying simply on the upregulation of a phase I pathway (CYP2B) as the creosote naive population did or a phase II pathway (GST) as the creosote experienced animals did may be an adequate response to the less volatile suite of PSC present in creosote.

4.2. Does the nasal epithelium respond to PSC in a similar manner as the liver?

Only *N. albigula* displayed a correlation between the expression of CYP2B and GST in the liver compared to the nasal epithelium. Again, this may be due to the volatile nature of juniper's PSC. As terpenes are so volatile, the inhaled PSC profile of juniper may be very similar to the ingested PSC profile. This relationship may cause the liver and nasal epithelium to have similar and therefore correlated responses. Interestingly both on control and 30% juniper diet, the *N. albigula* showed significant correlation between the liver and the nasal epithelium expression therefore even the constitutive expression of CYP2B and GST seemed to be linked between the livers and nasal epithelia of *N. albigula*.

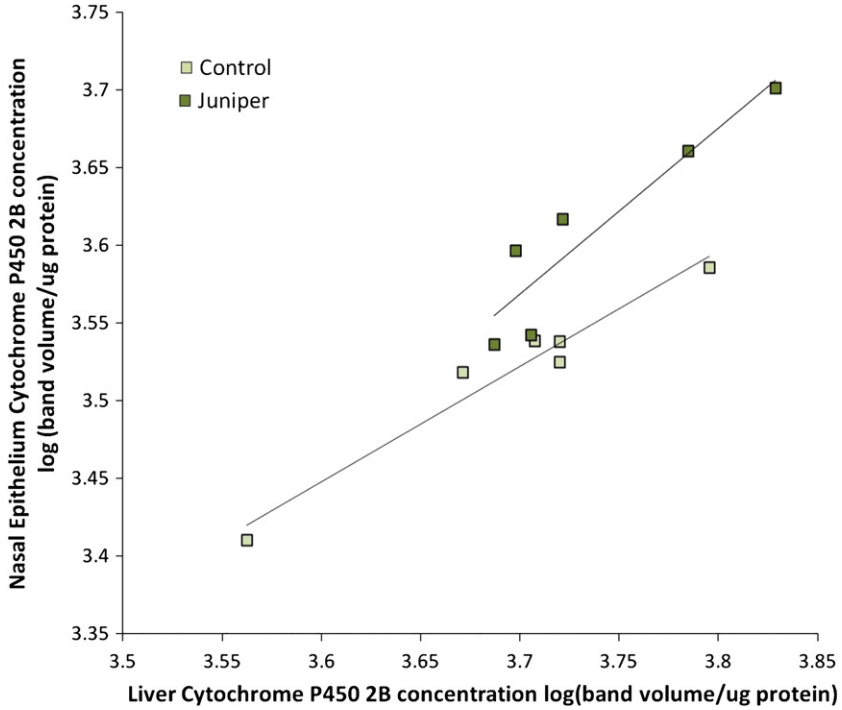

Fig. 5. Liver versus nasal epithelium CYP2B expression in *N. albigula* fed a control or 30% juniper diet. Liver expression of CYP2B significantly predicts nasal epithelium expression ($F_{1,8}=73.812$, $p<0.001$).

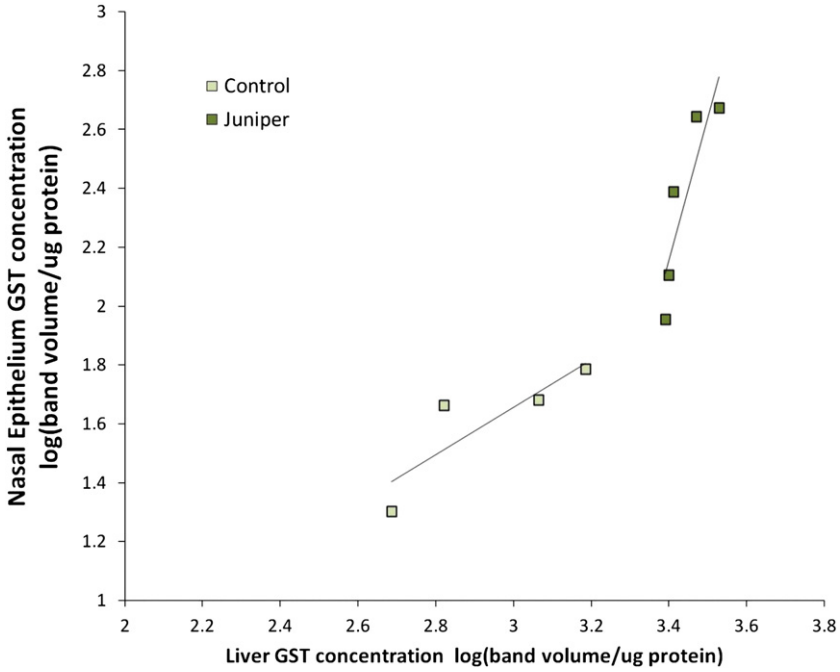

Fig. 6. Liver versus nasal epithelial GST expression in *N. albigula* fed a control diet or 30% juniper diet. Liver expression of GST significantly predicts nasal epithelial expression ($F_{1,5} = 15.970$, $p = 0.01$) but there is a difference between the two diet treatments ($F_{1,5} = 7.940$, $p = 0.037$). *N. albigula* fed the toxic diet have a significant correlation between liver and nasal epithelial expression of GST ($p = 0.047$) while the *N. albigula* fed the control did not ($p = 0.138$).

These data are particularly interesting in light of recent work demonstrating that the olfactory signal is modified by biotransformation enzymes in the olfactory epithelium, i.e., the perception of an odor is altered during biotransformation (Nagashima and Touhara, 2010). Woodrats reject diets high in PSC content while biotransformation enzymes are downregulated (pers. obs). Our feeding protocol includes gradually increasing the level of PSC in the diet over time to allow for upregulation of enzymes (Dearing et al., 2000; Mangione et al., 2000; Skopec et al., 2008; Torregrossa et al., 2011; Torregrossa et al., 2012). Once the necessary enzymes are induced the animals eat and maintain body mass on concentrations that would have been previously rejected (Dearing et al., 2000; Dearing et al., 2005b; Sorensen et al., 2005; Torregrossa et al., 2011; Torregrossa et al., 2012). The data presented here suggest that *N. albigula*'s induction of CYP2B and GST in the nasal epithelium is tightly correlated with the induction of the same enzyme in the liver. Perhaps, as the nasal epithelium induces enzymes, the perception of the odor changes and acts as an honest physiological signal of the liver's readiness. Although much more work must be done to test this hypothesis these data are a promising first step.

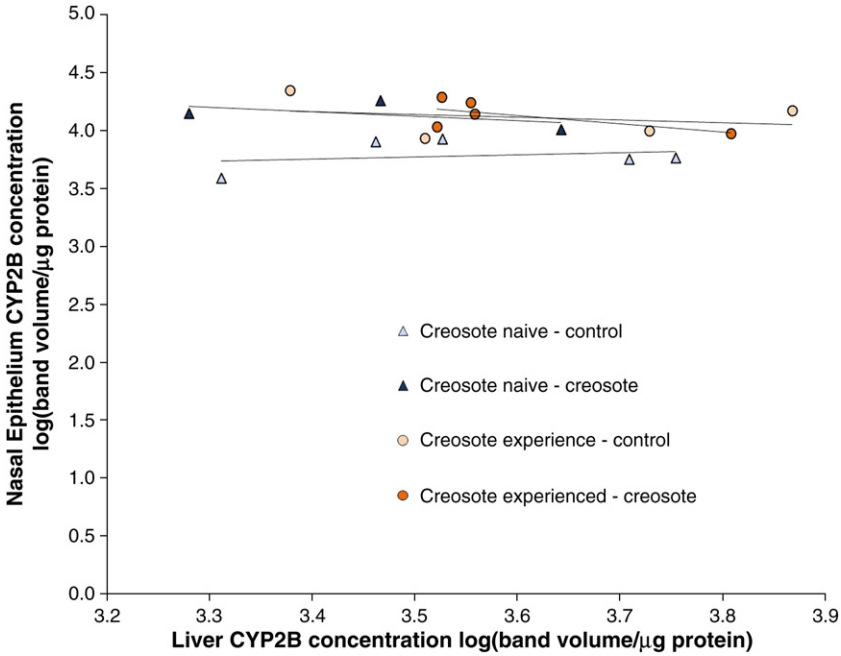

Fig. 7. Liver versus nasal epithelium CYP2B expression in *N. bryanti* that were creosote naïve or creosote experienced fed a control or a 2% creosote resin diet. There is no correlation between the liver and the nasal epithelial expression of CYP2B ($F_{1,12} = 0.662$, $p = 0.432$).

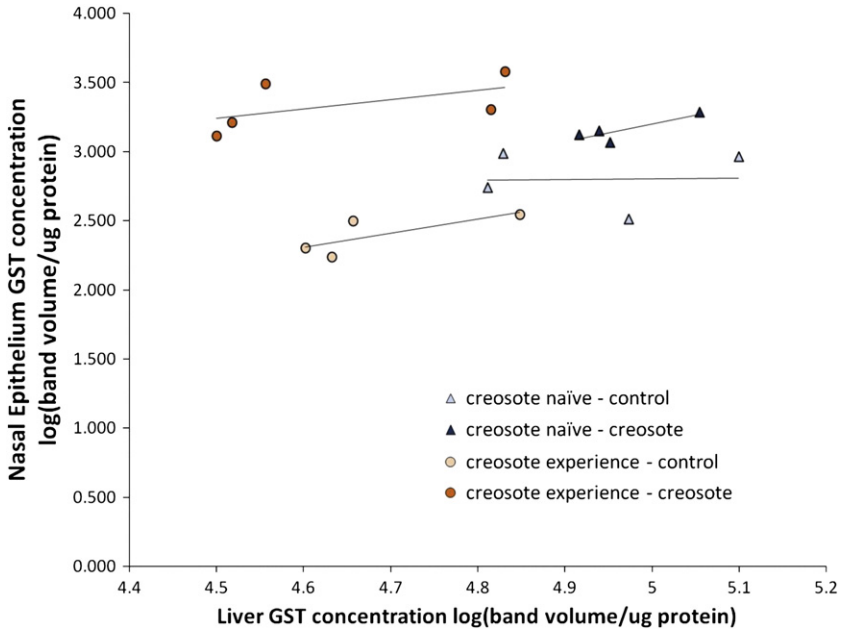

Fig. 8. Liver versus nasal epithelium GST expression in *N. bryanti* that were creosote naïve or creosote experienced fed a control or a 2% creosote resin diet. There is no correlation between the liver and the nasal epithelial expression of GST ($F_{1,12} = 3.021$, $p = 0.108$) in *N. bryanti*.

For *N. bryanti* as liver expression of CYP2B or GST increased there was little to no change in nasal epithelium expression of either enzyme constitutively or in response to a creosote diet. The suites of inhaled versus ingested PSC from the creosote diet versus the juniper diet likely differ given that phenolics are less volatile than terpenes, and this difference may have resulted in the lack of correlation in the responses of the liver and nasal epithelium when the woodrats were feeding on creosote. The creosote naive population of *N. bryanti* in the wild consumes a diet of oak and cactus, both of which contain mainly nonvolatile PSC like phenolics and oxalates (Atsatt and Ingram, 1983; Dearing et al., 2006; Haley et al., 2007a; Skopec et al., 2008) so similar to the creosote experienced *N. bryanti* there may not be an adaptive value to having correlation between the nasal epithelium and the liver expression of the same biotransformation enzymes.

Unlike the *N. albigula*, the toxins inhaled by *N. bryanti* feeding on creosote are not the same as the toxins that must be processed by the liver. Therefore we would not expect the upregulation of the same enzymes in the nasal epithelium. However, this does not necessarily refute the idea that the nasal epithelium could act to reflect liver readiness. It is possible that another enzyme is responsible for epithelial biotransformation and is upregulated in a similar time scale to those in the liver. Again, much more work would need to be done to directly address these hypotheses.

4.3. Is the response of the nasal epithelium an evolved or generalized response?

The two populations of *N. bryanti* had different physiological responses in both their livers and nasal epithelia in response to 2% creosote resin diets supporting the hypothesis that the response of the nasal epithelium is an evolved response. The creosote experienced animals had higher constitutive levels of CYP2B in their nasal epithelia compared to the creosote naive animals but the creosote naive animals upregulated CYP2B in response to creosote in the diet such that its nasal epithelium was expressing the same amount of CYP2B as the creosote experienced animals. The creosote experienced animals upregulated GST in their nasal epithelia when consuming creosote diets. Previous studies looking at the expression of biotransformation genes and enzymes as well as the activity of biotransformation enzymes in woodrat livers, intestine and/or kidneys have found that naive animals show different responses to PSC than experienced animals (Haley et al., 2007a,b; Skopec et al., 2007; Haley et al., 2008; Magnanou et al., 2009; Skopec and Dearing, 2011). It is therefore not surprising that the two populations of *N. bryanti* also showed differences in the expression of CYP2B and GST in response to creosote in their nasal epithelia. However these differences point to the importance of determining both species and population level specific susceptibilities to inhaled toxins.

5. Conclusions

To our knowledge this is the first study to investigate the expression of biotransformation enzymes in the nasal epithelium of wild caught herbivores. We found that the nasal epithelium responds to PSC present in the diet, that the response of the nasal epithelium was similar to the liver only in the *N. albigula* which regularly consume plants with volatile PSC, and that the response of the nasal epithelium is not a generalized response in that the expression of biotransformation enzymes differ in the nasal epithelium of animals that are experienced with the PSC profile of a plant versus animals that are naive to the plant.

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